Stomatal Density and Index Analysis of Fossilized Pliocene Floral Remains from Ellesmere Island, Canada For the Identification of Ancient Carbon Dioxide Levels

Senior Thesis
Submitted in partial fulfillment of the requirements for the
Bachelor of Science Degree
At The Ohio State University

By

Wesley J. Koewler
The Ohio State University
2016

Approved by

Dr. Joel Barker, Advisor
School of Earth Sciences

Table of Contents

ABSTRACT

The Earth's climate is changing due in large part to increasing concentrations of greenhouse gases in the atmosphere. Climate modeling suggests that atmospheric CO_2 concentration may reach 1000 ppm, and the global average temperature may increase on the order of 2–3 °C over the next century. Despite a high degree of confidence in these predictions on a global scale, regional scale climate effects are less certain, particularly for polar regions. To help reconcile this uncertainty, Pliocene-aged deposits are being examined to help identify the consequence of relatively high atmospheric CO_2 concentrations in the Arctic because the concentration of CO_2 in the atmosphere during the Pliocene is within the range of those concentrations predicted for the next 75 years. One proxy for past atmospheric CO_2 is the density of stoma in the fossilized leaves of Pliocene-aged plants.

This study uses mummified needles from a Pliocene-aged deposit in the Arctic to estimate atmospheric CO_2. Results indicate a 45.07% decrease in stomatal density from current needles, corresponding to an atmospheric CO_2 concentration of ~338 ppmV. This concentration is lower than the present day, and lower than CO_2 concentrations for much of the Pliocene, suggesting a late Pliocene age for the deposit.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to some of the people that have journeyed with me throughout the entire thesis process. First and foremost, I must thank Dr. Joel Barker, my research advisor, for taking me under his wing and helping me in creating this thesis. I was never the most academically inclined student, and with his help and patience I was able to undertake this rigorous assignment and see it through to the end. I also need to thank Dr. Anne Carey and Dr. Karen Royce for also working alongside me in this process. I also want to thank Dr. Ellen Currano and her grad student Amanda Patrick at the University of Wyoming for taking my samples and imaging them for me. I also extend my thanks to the entirety of The School of Earth Sciences here at The Ohio State University. Also thank you to NSF Division of Polar Programs grant 1026177 for supplying the funding used to collect the samples. Thank you to my mother, father, and sister, as well as my friends who never stopped pushing me and wishing me the best of luck. Last but not least I would like to my best friend Rachel for setting an amazing example, and leading me home when I am lost. Thank you!

INTRODUCTION

The global climate is changing at an alarming rate. According to the Intergovernmental Panel on Climate Change (IPCC) the last decade of the 20th century and the first two decades of the 21st century have been the warmest since 1850, when instrumental temperature records started to be collected (Pachauri et al,, 2014). This increase in temperature is linked to an increase in human induced carbon dioxide (CO_2), and other greenhouse gas (GHG) emissions. CO_2 was the dominant GHG emitted from 1970 to 2010, contributing 78% to the total GHG flux to the atmosphere (Pachauri et al,, 2014). The increase of GHGs in the atmosphere results in an overall warmer atmosphere, and warmer global surface temperatures (Fig. 1; Karl et al., 2003).

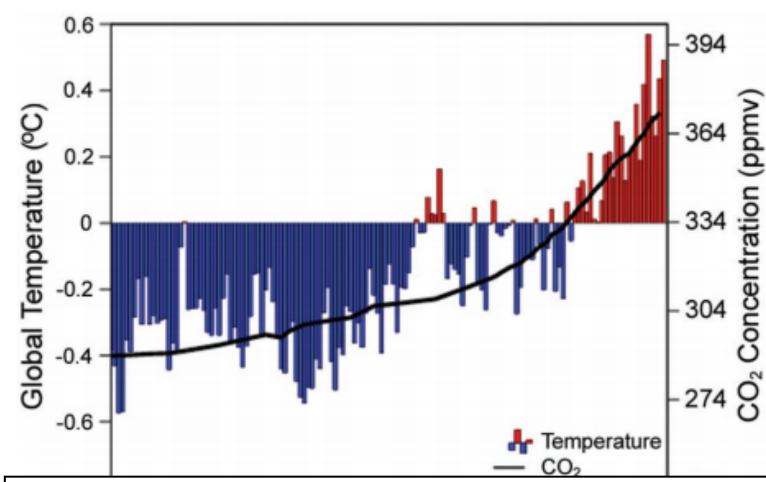

Fig 1. Chart showing correlation between atmospheric CO_2 levels and mean global temperature. Time series of departures from the 1961 to 1990 base period for an annual mean global temperature of 14.0°C (blue bars mean below, red mean above the mean)

As of early 2016, atmospheric CO_2 levels are 404.02 parts per million (ppm) according to the latest reading from the Mauna Loa observatory (http://www.esrl.noaa.gov/gmd/ccgg/trends/index.html). According to IPCC projections, atmospheric CO_2 concentrations may surpass 1000 ppm by the end of this century (Pachauri et al., 2014). The resultant temperature change is projected to be at least ~2-3 °C over the same time period (Pachauri et al., 2014).

The last time in Earth's history that the concentration of atmospheric CO_2 exceeded 400 ppm was during the Pliocene epoch, 5-2.6 million years ago (Ma). Consequently, the Pliocene-aged deposits are being examined for clues to predict changes that might be result under future CO_2 concentration scenarios (Martinez-Boti et al., 2015). Current knowledge of the Pliocene epoch comes mainly from regression models based on marine foraminifera where climate forcing and temperature are estimated within a restricted interval to calculate what the temperature was likely to have been over a specific time interval (Martinez-Boti et al., 2015). Results suggest that globally, the Pliocene was likely 2–3 °C warmer than today.

Despite an understanding of global temperature and atmospheric CO_2 concentration during the Pliocene, local and regional temperature and climate are largely unknown and may differ significantly from the global average. For example, phenomena such as "polar amplification" (Holland and Bitz, 2003) would result in an increased rate and amplitude of temperature increase in the Arctic in response to changing atmospheric CO_2 relative to the global average. Therefore, it is important to resolve polar region CO_2 and temperature during the Pliocene to better predict future changes in the Arctic.

One proxy that is used to determine past atmospheric CO_2 is the density of leaf stoma in fossilized terrestrial plants. This technique examines the inverse relationship that exists between the level of atmospheric CO_2 and the stomatal density of some plants (Van de water et al., 1994; Lin et al., 2001; Royer, 2001; Konrad et al., 2008; Franks et al., 2012; Franks et al., 2013; Haworth et al., 2015; Lomax and Fraser, 2015).

During the relatively warm Pliocene, the northern hemispheric treeline extended north to the Arctic Ocean, approximately 2000 km further north than current treeline. Several Pliocene-aged forest deposits have been preserved via desiccation (mummification) as sedimentary deposits in the Arctic. Within these sedimentary deposits are foliage samples that could prove to be useful for estimating atmospheric CO_2 levels during the Pliocene and in doing so improve predictions on changes that can be anticipated for Arctic regions during future CO_2 increases and associated climate change.

Here, we examine the density of stomata in leaves from *Pinus* (pine) and *Larix* (larch) needles recovered from an Arctic fossil forest site. Further, we explore the viability of using the stoma in mummified pine and birch leaves from this deposit to deduce Arctic atmospheric CO_2 during the Pliocene.

Field Site and Methods

Bulk samples were taken from a fossil forest deposit on Ellesmere Island, Canada, in the summer of 2010 (81° 40’ N, 76° 14’ W; Fig. 2).

Figure 2. Map showing location of the sedimentary deposit where samples were collected from on Ellesmere Island (taken from Google earth)

The deposit is thought to be Pliocene-aged based on the plant species present and compared to other Pliocene-aged deposits in the Arctic (Barker, pers. comm.). Samples from the deposit were transported to the Byrd Polar and Climate Research Center at The Ohio State University in containers that minimized exposure to air and light.

A total of 75 *Larix* spp. (larch; Fig. 3) and 98 *Pinus* spp. (pine; Fig. 4) needles were identified visually using their morphological characteristics using an Olympus SZ61 stereo zoom dissection microscope. After the needles were sorted by species, the best preserved of each type, 26 of each, were placed into small glass jars and transported to Dr. Ellen Currano at the University of Wyoming. Individual needles were illuminated under a Nikon LV100 compound microscope using Sola light engine SM II LE and an Endow GFP longpass green filter (exciter HQ470/40 ex, dichroic 495, emitter 500LP).

Stomatal density was calculated from the fluorescence microscopy imagery when it was possible to identify stomates in the sample needles. Stomatal density is determined by measuring number of stomates visible per unit area of the needle. For the larch samples, stomata number per length is also calculated. This is due to the fact that larch needles only have a single row of stomata per needle. Stomatal index is then calculated by dividing the sum of stomatal densities by the epidermal cell density and expressed as a percentage. Stomatal index and density can then be compared to existing calibration curves where the individual plant species' response to changing CO_2 levels has been determined (Royer, 2001).

Figure 3. Sample of a pine needle.

Figure 4. Sample of a larch needle.

Results

Of the 52 needles examined by fluorescence spectroscopy, only 1 was preserved sufficiently to permit the identification of individual stoma and epidermal cells (Fig. 5). The other samples did not provide any useful information due to their advanced stage of degradation (Fig. 6 and 7).

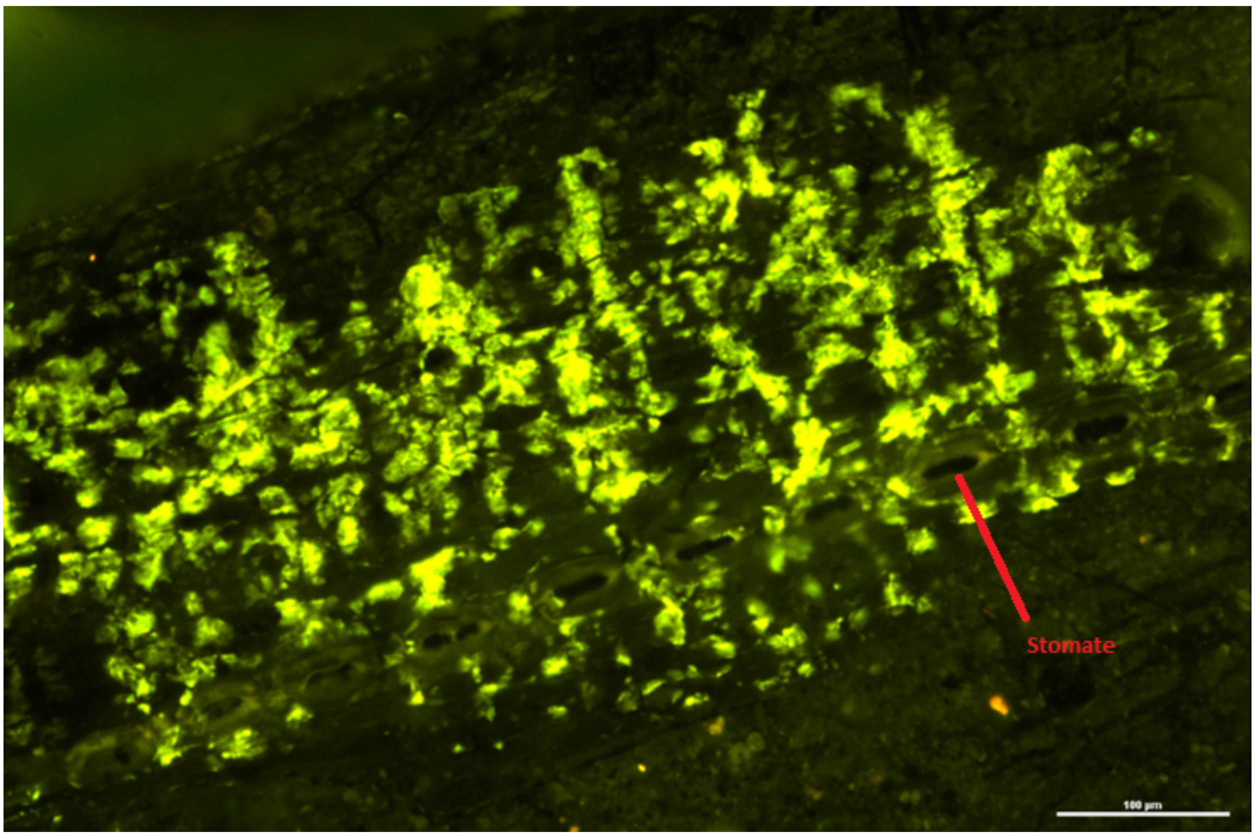

Figure 5. Fluorescent microscopy image of larch needle (only viable photo). Stomata are in a row on the surface, and everything else around it is epidermis. The scale bar is 100 μm.

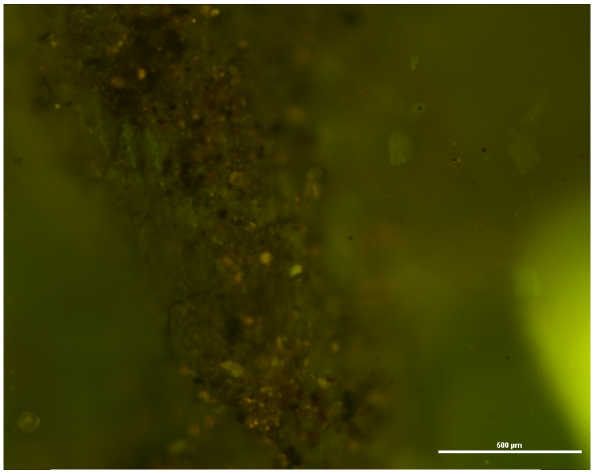

Figure 6. Typical pine sample showing no stoma. Scale bar is 500 μm

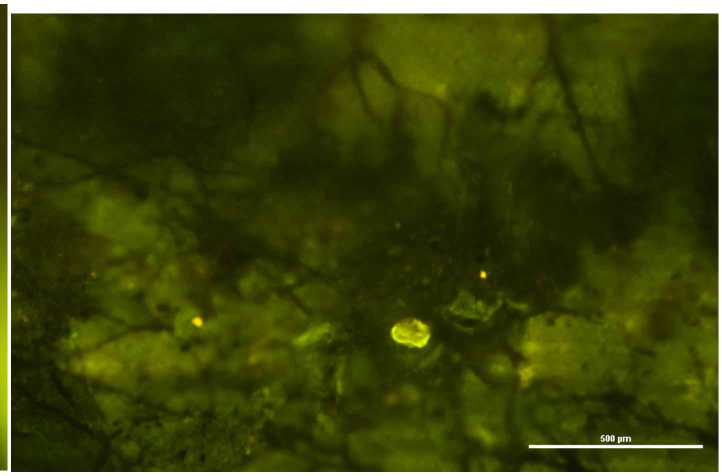

Figure 7. Typical larch sample showing no stoma. Scale bar is 500 μm

Stomatal density was calculated out to be 36.53 mm^{-2}. Also calculated was the stomata per length (in mm) of the larch needle, which was 13.33 stomata mm^{-1}. The single useful image does not show any differentiation between epidermal cells, which does not allow for the calculation of a stomatal index.

Kouwenberg et al. (2003) use stomata number per millimeter rather than stomatal density or index, specifically for larch and other conifers that have a single row of stomata per leaf.

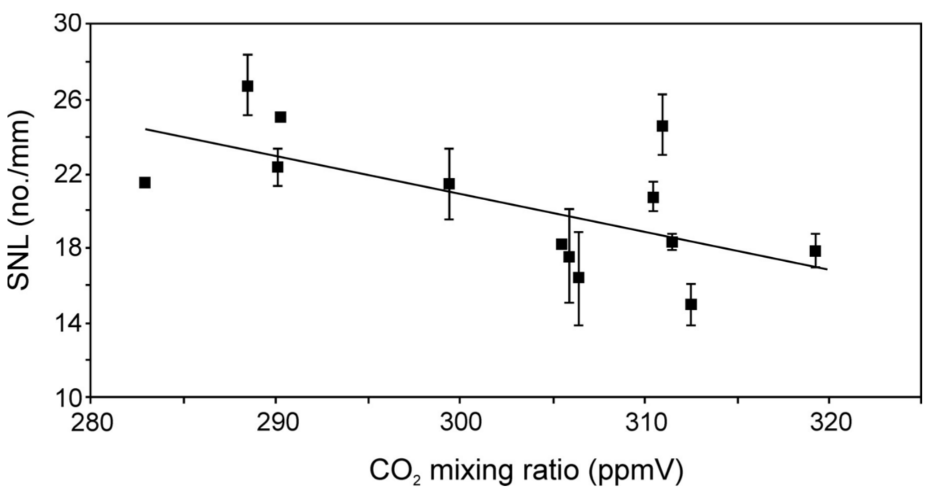

Figure 8. Graph relating stomata number per length (SNL; mm^{-1}) relative to CO_2 mixing ratio (the amount of CO_2 per total volume of atmospheric gas measured) in *Larix larcina* (Kouwenberg et al., 2003). The slope of the regression line is. -0.1983 (r^2 = 0.3884).

Discussion

The stomatal density of modern larch needles (CO_2 at 380 ppmV) is 66.5 mm^{-2} (Wang et al., 2006). The stomatal density of the mummified larch needle is 45.07% less than the present day larch needles.

Figure 8 shows the stomatal response to ambient CO_2 concentrations in larch (*Larix larcina;* Kouwenberg et al., 2002). With the slope of the regression line and an assumption that the linear trend can be extended beyond the scale presented, the atmospheric CO_2 level of 338.29 ppmV corresponds to the measured SNL of 13.33 in the Pliocene larch needle. During the latter part of the Pliocene (near the Pliocene-Pleistocene transition) atmospheric CO_2 concentration decreased to these levels (Franks et al., 2014) suggesting that the Arctic Pliocene deposit is late Pliocene in age. The stomatal density levels of the larch cannot currently be linked to an explicit quantity of atmospheric CO_2 because it was difficult to find a portion of the needle that was completed intact (Fig. 5). While the stoma were easily identified, the surrounding epidermis was degraded.

This result is derived from a single sample because all of the other samples were too degraded to provide stomatal abundance. Consequently, we cannot provide any measure of confidence in this result. However, the facts that a) our calculated value of 338.29 ppmV falls within the range of values reported for the late Pliocene as conditions were cooling into the Pleistocene glacial period, and b) larch is a northern boreal tree species adapted to cool temperatures, as would coincide with low atmospheric CO_2 concentration, lead us to believe that our estimate of atmospheric CO_2 and corresponding allocation of a late Pliocene age to the deposit is plausible.

Conclusions

The main objective of this research was to determine whether or not mummified larch and pine needle remains from a Pliocene deposit on Ellesmere Island, Canada could function as a proxy for atmospheric CO_2 during that epoch. That objective was partially realized in that the single usable sample provided data that indicated CO_2 levels similar to what has been estimated for the late Pliocene epoch, close to the Pliocene-Pleistocene transition. Our objective could have been more fully realized if the degradation of the needles sampled was not so severe as to prevent stomatal index calculations to be made using more larch and pine samples. It would have also been easier to verify the data and quantify the confidence of our CO_2 estimate if there had been several more useful samples. While the effectiveness of larch needles as a proxy for atmospheric CO_2 levels during the Pliocene cannot be easily evaluated here, it seems that our estimate of Arctic Pliocene CO_2 is plausible for the late Pliocene.

Recommendations for Future Work

To extend the data and research presented in this paper, more samples from the Ellesmere Island deposit that have been preserved sufficiently well need to be analyzed. The fluorescence spectroscopy-based analytical procedure used here appears to work well to provide a usable larch sample showing stoma needed to determine stomatal density, SNL, and potentially, stomatal index. Once multiple samples showing stoma have been found they can be used to verify whether or not the mummified remains provide a good source of proxy data to be used in the coming century.

References Cited

Anon (n.d.), ESRL Global Monitoring Division - Global Greenhouse Gas Reference Network, *ESRL CO_2 Trends RSS*. Available from: http://www.esrl.noaa.gov/gmd/ccgg/trends/index.html (Accessed 4 April 2016)

Franks, P. J., I. J. Leitch, E. M. Ruszala, A. M. Hetherington, and D. J. Beerling (2012), Physiological framework for adaptation of stomata to CO_2 from glacial to future concentrations, *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1588), 537–546.

Franks, P. J., M. A. Adams, J. S. Amthor, M. M. Barbour, J. A. Berry, D. S. Ellsworth, G. D. Farquhar, O. Ghannoun, J. Lloyd, N. Mcdowell, R. J. Norby, D. T. Tissue, S. V. Caemmerer (2013), Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century, *New Phytologist New Phytol*, *197*(4), 1077–1094.

Franks, P. J., D. L. Royer, D. J. Beerling, P. K. V. D. Water, D. J. Cantrill, M. M. Barbour, and J. A. Berry (2014), New constraints on atmospheric CO_2 concentration for the Phanerozoic, *Geophysical Research Letters*, *41(13)*, 4685–4694.

Haworth, M., D. Killi, A. Materassi, and A. Raschi (2015), Coordination of stomatal physiological behavior and morphology with carbon dioxide determines stomatal control, *American Journal of Botany*, *102*(5), 677–688.

Holland, M. M., and C. M. Bitz (2003), Polar amplification of climate change in coupled models, *Climate Dynamics*, *21*(3-4), 221–232.

Karl, T. R. (2003), Modern Global Climate Change, *Science*, *302*(5651), 1719–1723.

Konrad, W., A. Roth-Nebelsick, and M. Grein (2008), Modelling of stomatal density response to atmospheric, *Journal of Theoretical Biology*, *253*(4), 638–658.

Kouwenberg, L. L. R., J. C. Mcelwain, W. M. Kurschner, F. Wagner, D. J. Beerling, F. E. Mayle, and H. Visscher (2003), Stomatal frequency adjustment of four conifer species to historical changes in atmospheric CO_2, *American Journal of Botany*, *90*(4), 610–619.

Lin, J., M. E. Jach, and R. Ceulemans (2001), Stomatal density and needle anatomy of Scots pine (*Pinus sylvestris*) are affected by elevated CO_2, *New Phytologist New Phytol*, *150*(3), 665–674.

Lomax, B. H., and W. T. Fraser (2015), Palaeoproxies: botanical monitors and recorders of atmospheric change, *Palaeontology*, *58*(5), 759–768.

Martínez-Botí, M. A., G. L. Foster, T. B. Chalk, E. J. Rohling, P. F. Sexton, D. J. Lunt, R. D. Pancost, M. P. S. Badger, and D. N. Schmidt (2015), Plio-Pleistocene climate sensitivity evaluated using high-resolution CO_2 records, *Nature*, *518*(7537), 49–54.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., Church, J.A., Clarke, L., Dahe, Q., Dasgupta, P., Dubash, N.K., Edenhofer, O., Elgizouli, I., Field, C.B., Forster, P., Friedlingstein, P., Fuglestvedt, J., Gomez-Echeverri, L., Hallegatte, S., Hegerl, G., Howden, M., Jiang, K., Jimenez Cisneroz, B., Kattsov, V., Lee, H., Mach, K. J., Marotzke, J., Mastrandrea, M.D., Meyer, L., Minx, J., Mulugetta, Y., O'Brien, K., Oppenheimer, M., Pereira, J.J., Pichs-Madruga, R., Plattner, G.K., Pörtner, H.O., Power, S.B., Preston, B., Ravindranath, N.H., Reisinger, A., Riahi, K., Rusticucci, M., Scholes, R., Seyboth, K., Sokona, Y., Stavins, R., Stocker, T.F., Tschakert, P., van Vuuren, D., van Ypserle, J.P. (2014), Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland, IPCC, 151 p., ISBN: 978-92-9169-143-2.

Royer, D. (2001), Stomatal density and stomatal index as indicators of paleoatmospheric CO2 concentration, *Review of Palaeobotany and Palynology*, *114*(1-2), 1–28.

Van De Water, P. K., S. W. Leavitt, and J. L. Betancourt (1994), Trends in Stomatal Density and $^{13}C/^{12}C$ Ratios of *Pinus flexilis* Needles During Last Glacial-Interglacial Cycle, *Science*, *264*(5156), 239–243, doi:10.1126/science.264.5156.239.

Wang, W., Y. Zu, S. Cui, T. Hirano, Y. Watanabe, and T. Koike (2006), Carbon dioxide exchange of larch (*Larix gmelinii*) cones during development, *Tree Physiology*, *26*(10), 1363–1368.